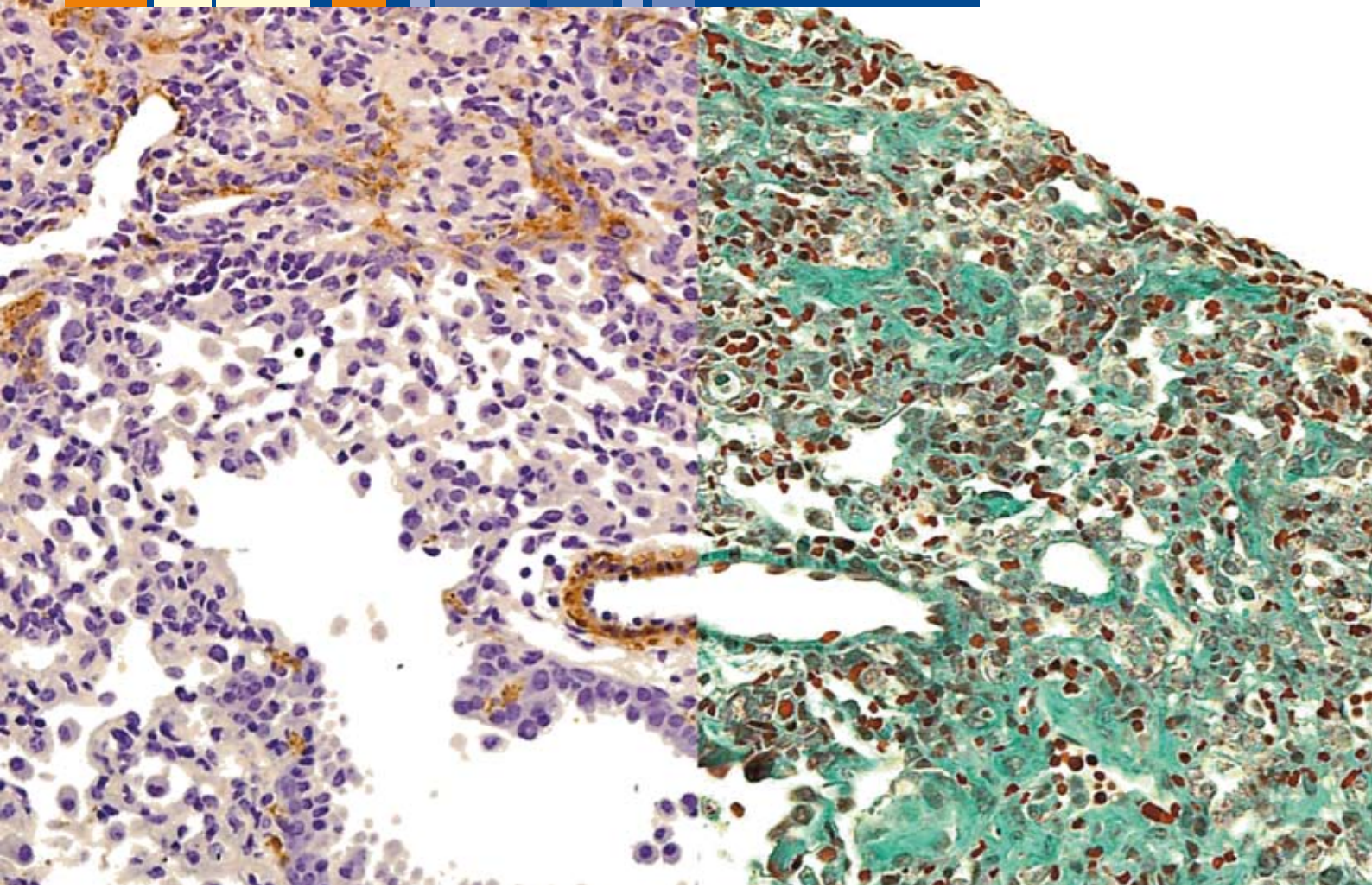
In vivo and *in vitro* models for fibrosis research

TNO | Knowledge for business



Mouse lung at 14 days after bleomycin-induction showing α-smooth muscle actin expressing myofibroblasts (brown, left) and collagen accumulation (right, stained green using Masson's Trichrome staining).

Currently, there are not any approved therapies that directly target fibrosis. The lack of therapeutic options and the enormous impact fibrosis has within various diseases, presents both a challenge and a great opportunity for companies working to develop therapies for fibrosis. TNO has a portfolio of *in vivo* and *in vitro* models of fibrosis and the expertise to support you during the development of your anti-fibrotic compound.

TNO services

TNO has developed an extensive and unique portfolio of fibrosis models. These tools support the validation of new targets, the testing and optimization of new treatments and give insight into the disease mechanism. We offer a range of *in vivo* models that mimic the fibrosis process in a number of organs. Additionally, we can provide you with a series of *in vitro* models based on primary human cells. These *in vitro* models are specifically suited to investigate processes within the fibrotic pathway.

Our fibrosis research focuses on the following organs:

- Lung
- Skin
- Liver
- Kidney

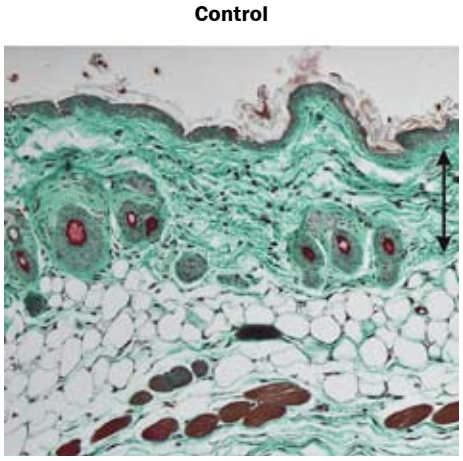


Figure 1. Histology of bleomycin-induced skin fibrosis in mice. Trichrome Masson staining. Top: normal skin. Bottom: Fibrotic skin showing accumulation of collagen (stained green).

Bleomycin-induced lung fibrosis

To study novel compounds against idiopathic pulmonary fibrosis (IPF), we established the bleomycin-induced lung fibrosis model in mice (model in rat is being validated). This model is induced by the intratracheal instillation of clinical grade bleomycin into the lungs of C57Bl/6 mice. This induces aberrant myofibroblast differentiation accompanied by severe collagen deposition in the alveolar spaces in several weeks. We can investigate different aspects of the fibrotic process by studying this model at various time points. We can test your anti-fibrotic compound either in a prophylactic or in a therapeutic treatment regimen using histology and collagen content as read-outs. We are currently developing functional read-outs focusing on respiratory and exercise parameters.

During the first week after induction, this model is also used as an acute inflammatory model using cytokine content in the broncho-alveolar lavage (BAL) or influx of inflammatory cells (BAL or histology) as read-outs.

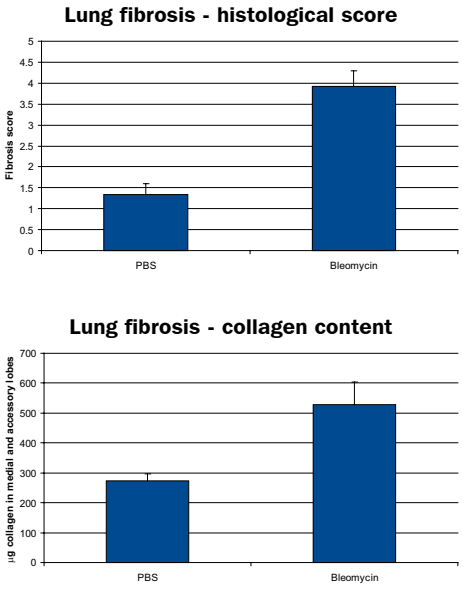


Figure 2. Induction of lung fibrosis in mice by the intratracheal instillation of bleomycin results in a strong increase in histological fibrosis score (top) and collagen content of the lung lobes (bottom).

Bleomycin-induced skin fibrosis

One of the features of systemic scleroderma is the development of skin fibrosis. To evaluate novel anti-fibrotic therapeutics, we offer the bleomycin induced skin fibrosis mouse model. In this model, C57Bl/6 mice receive repetitive subcutaneous bleomycin injections into the neck area. This results in dermal thickening and collagen accumulation over a period of five weeks. The main read-outs are skin thickness (image analysis of Masson's trichrome stained histological sections, see figure 1) and collagen content of the skin.

Liver fibrosis

Liver fibrosis is the ninth leading cause of death in the world. We offer a model for liver fibrosis based on induction by repeated carbon tetrachloride (CCL_4) administration into C57Bl/6 mice. In this model, liver fibrosis is present after 4 weeks, as characterized by the accumulation of collagen and the presence of α-smooth

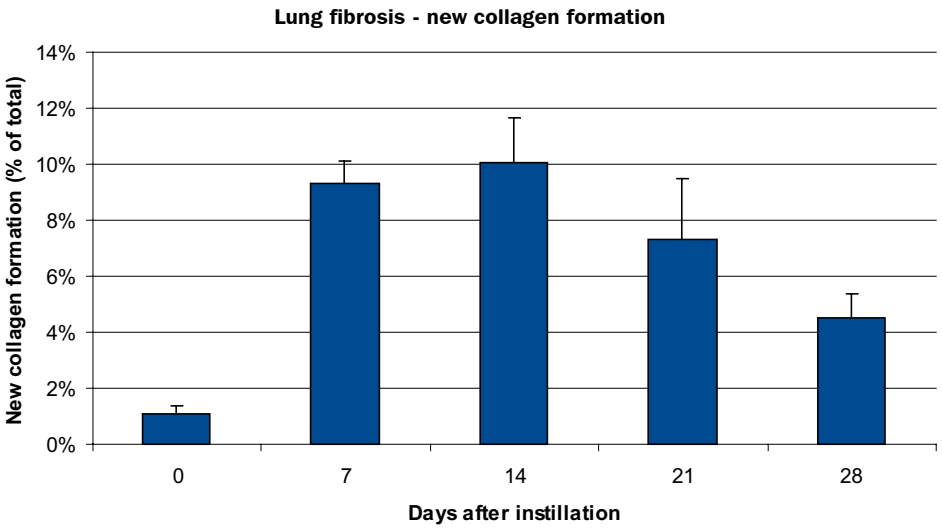


Figure 3. New collagen formation was determined using deuterized water labeling during one week before sacrifice.

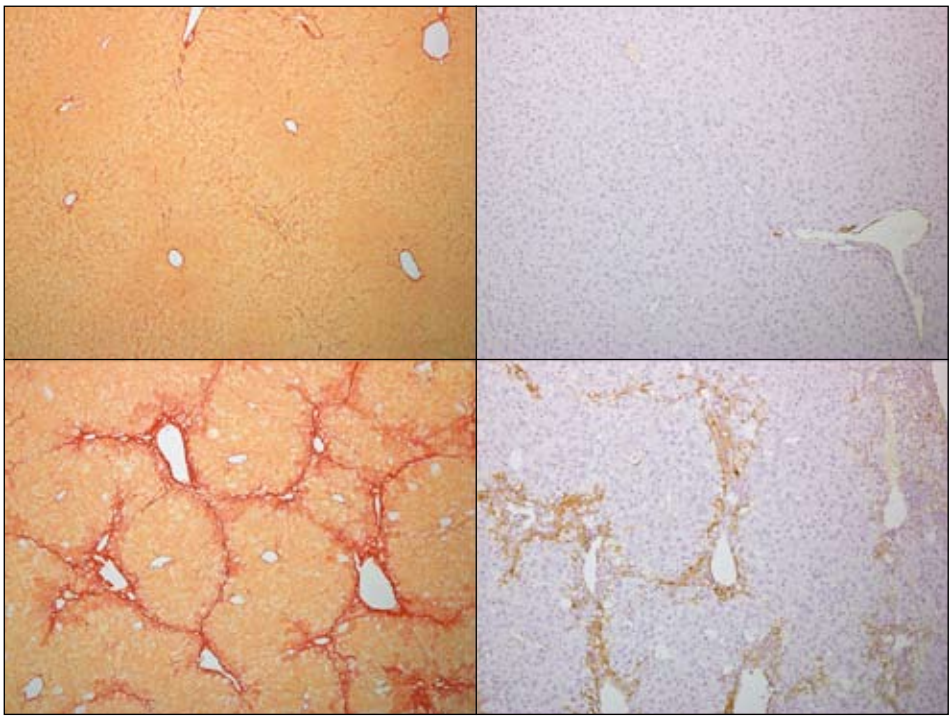


Figure 4. CCL_4-induced liver fibrosis results in increased collagen formation and myofibroblast differentiation. Top: control animals; bottom: CCL_4-induced. Left: collagen (Sirius red staining); right (α-SMA expression).

muscle actin positive cells (see figure 4). The collagen content of liver lobes as determined by HPLC and image analysis of histological slides are used as read-outs.
In collaboration with the department of metabolic diseases we are developing liver injury models related to metabolic syndrome and non-alcoholic steatohepatitis (NASH).

Kidney fibrosis

Kidney fibrosis is a rapidly developing area in fibrosis research. A major part of our current development program focuses on the establishing and optimizing models for kidney injury (ischaemic reperfusion injury, glomerulonephritis), diabetic nephropathy and kidney fibrosis (unilateral urether obstruction).

In vitro fibrosis

Due to the complexity of the *in vivo* fibrosis process, it is very difficult to mimic this process with a single *in vitro* assay. Therefore, we offer a set of *in vitro* assays, each representing key processes within the fibrotic pathway, such as myofibroblast differentiation, profileration, migration, and collagen production. These assays, based on primary human cells, are well-suited to evaluate effects of compounds on human targets.

Customized study design

All the studies using the models in this information sheet can be customized to suit your specific needs. For example, we offer different routes of administration (oral, i.p., s.c., i.v., or topical). We can provide both prophylactic and therapeutic dosing regimes and have a wide range of optional read-outs. To determine specific aspects of your compound's characteristics in our models, our additional read-outs include histology (specific staining, for example focusing on infiltration of inflammatory cells), immunohistochemistry (α-SMA as a marker for myofibroblasts, BrdU as marker for dividing cells, etc.), biochemical analyses (collagen content, collagen modifications, etc) and gene expression analysis.
An exciting new development within our portfolio is the use of deuterized water to specifically label and quantify collagens formed during fibrosis, allowing easier and more robust detection of drug-induced changes (figure 3).

Complementary expertise

TNO also offers a broad range of models and expertise in related disease areas, including inflammation, liver steatosis, wound healing, and angiogenesis and organs including the lung, skin and liver.

Lung

TNO has extensive experience in the field of inhalation toxicology, based on exposure with potentially toxic substances by breathing them. Studies can vary from a single administration to chronic exposure. We have experience generating, monitoring and analyzing the chemical and physical behavior of test atmospheres with a wide range of test materials. Our inhalation facilities are equipped with state-of-the-art generation and monitoring techniques. Our specialty is providing well-controlled test atmospheres for challenging test substances.

Skin

TNO has developed unique expertise with regard to (human) skin. In addition to a unique humanized model of psoriasis and a mouse model of atopic dermatitis, we have expertise concerning skin safety models, as well as the use of skin as a target for drug delivery.

Liver

TNO has a broad expertise in liver steatosis. We can study the effects of your compounds on a diverse array of processes including lipid accumulation, inflammation, identification of molecular targets and pharmacokinetics.

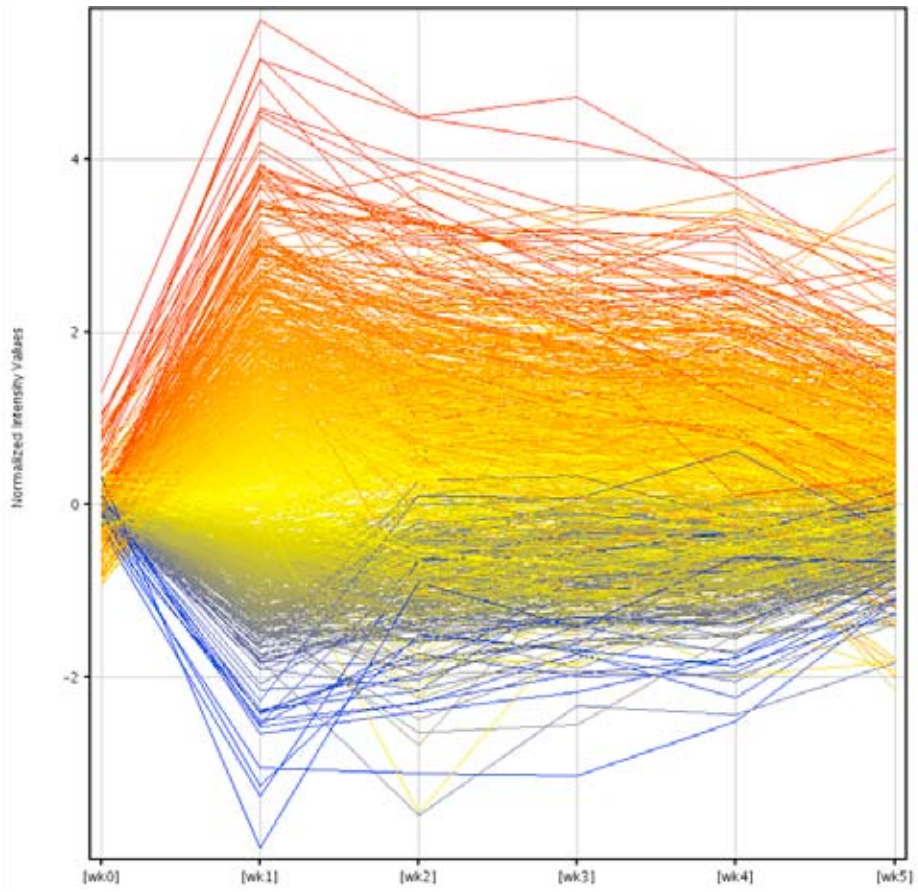


Figure 5. Total mRNA expression during a time-course of mouse lung fibrosis: array analysis.

Wound healing/angiogenesis models

To study the effects of compounds on both the stimulation and inhibition of wound healing, we offer models in both normal and diabetic mice. We offer wound closure kinetics, but can also measure angiogenesis using laser doppler analysis as read-out. In another angiogenesis model, we run the mouse angioreactor model which allows us to investigate the effect (anti- and pro-angiogenic) of compounds on blood vessel formation.

Actively seeking co-development partners

We are continuously expanding and improving our fibrosis portfolio, focusing on three areas: 1) new animal models for fibrosis, 2) the development of more translational and predictive read-outs, and 3) an on depth understanding of the processes occurring in fibrosis. To ensure that our portfolio matches your needs, we are actively seeking partners in these areas.
These developments include: (new) models for kidney fibrosis, functional read-outs for lung and liver fibrosis models, such as respiratory and exercise analysis, and extracellular matrix remodeling and collagen cross-linking. If you are considering working in these areas, we would like to discuss the mutual advantages of collaborating.

TNO Quality of Life

TNO Quality of Life is a part of TNO; Europe's largest independent research institute for technological and strategic research and consultancy. By translating scientific knowledge into practice we optimise the innovative abilities of the industry and government. As a research partner TNO works for various industries worldwide.

Visiting address
Zernikedreef 9
2333 CK Leiden
The Netherlands

Postal address
P.O. Box 2215
2301 CE Leiden
The Netherlands

For more information, please contact us.
E info-pharma@tno.nl

The Netherlands, Leiden
P +31 71 518 14 99
F +31 71 518 19 01

The Netherlands, Zeist
P +31 30 694 47 41
F +31 30 694 48 45

North America
P +1 416 837 75 00
F +31 71 518 19 01

Japan
P +81 45 478 51 30
F +81 45 473 79 59

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